

Complete Genome Sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* Strain ND02[∇]

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Received 6 April 2011/Accepted 13 April 2011

***Lactobacillus delbrueckii* subsp. *bulgaricus* strain ND02 is a Chinese commercial dairy starter used for the manufacture of yoghurt. It was isolated from naturally fermented yak milk in Qinghai, China. Here, we report the main genome features of ND02 and several differences with two other published genomes of *Lactobacillus delbrueckii* subsp. *bulgaricus* strains.**

Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* are the most important strains used for industrial dairy starters. *L. delbrueckii* subsp. *bulgaricus* strain ND02 was isolated from naturally fermented yak milk in Qinghai, China (7). It has many excellent processing properties, such as moderate acidity, high viscosity, and water holding (7). This strain has been implemented in the industrial production of dairy starter cultures by Inner Mongolia Yili Industrial Group Company Limited, the largest dairy corporation in China.

Whole-genome sequencing of *L. delbrueckii* subsp. *bulgaricus* strain ND02 was performed with a combined strategy of 454 sequencing (6) and Solexa paired-end sequencing technology (1). Genomic libraries containing 8-kb inserts were constructed, and 62,098 paired-end reads and 127,000 single-end reads were generated using the GS FLX system, giving 28.0-fold coverage of the genome. A portion (95.3%) of these reads was assembled into two large scaffolds, including 283 non-redundant contigs, using the 454 Newbler assembler (454 Life Sciences, Branford, CT). A total of 8,778,388 reads (2.5-kb library) were generated to reach a depth of 218-fold coverage with an Illumina Solexa GA IIx (Illumina, San Diego, CA) and mapped to the scaffolds using Burrows-Wheeler Aligner (BWA) (4). The gaps between scaffolds were filled by sequencing PCR products using an ABI 3730 capillary sequencer. The genome analysis was performed as described previously (2, 3).

The complete genome sequence of ND02 contains a circular 2,125,753-bp chromosome and a 6,223-bp plasmid named LDBND_P, with mean GC contents of 49.56% and 44.66%, respectively. There are 2,177 genes in total, including 2,012

coding genes, 44 pseudogenes, 9 rRNA operons, and 94 tRNAs in the chromosome, as well as 6 coding genes in the plasmid.

Comparison of ATCC 11842 (9), ATCC BAA-365 (5), and ND02 genomes revealed that they were highly similar, with the exception of 416 encoding genes that are uniquely present in ND02 but not in the other two strains. Some of the unique genes formed six large insertion islands that were comprised by transposase, glutamate decarboxylase, acetyltransferase, glycosyltransferase, alcohol dehydrogenase, polysaccharide biosynthesis protein, and an exopolysaccharide (EPS) biosynthesis gene cluster.

Similar to the other two *L. delbrueckii* subsp. *bulgaricus* strains, ND02 partially inactivates several sugar transport and degradation pathways, with a preference for lactose in carbohydrate metabolism. Although some of the putative transporters for ribose (LDBND_0298) and ribokinase (LDBND_0152) are present in the genome, ND02 cannot grow on ribose as the only carbon source, presumably as a consequence of an incomplete pentose-phosphate pathway. Interestingly, a unique putative protein of raffinose permease (LDBND_1126) and raffinose operon transcriptional regulatory protein (RafR, LDBND_1126) are found in the genome of ND02 (without raffinose-specific PTS) by comparison of these three genomes. ND02 can grow on raffinose as the only carbon source, whereas the other two strains cannot grow (data not shown). This may be due to the raffinose permease of ND02, which was allosterically regulated for the uptake of non-PTS carbohydrates as a permease for lactose, maltose, and melibiose (8).

Nucleotide sequence accession numbers. The sequence and annotation of the *Lactobacillus delbrueckii* subsp. *bulgaricus* ND02 chromosome and plasmid are available from GenBank under accession numbers CP002341 and CP002342, respectively.

This research was supported by the National Natural Science Foundation of China (no. 31025019), the Earmarked Fund for Modern Agro-industry Technology Research System, the Prophase Research

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∇ Published ahead of print on 22 April 2011.

Program of the 973 Project of China (2010CB134502), the National Key Technology R&D Program (no. 2009BAD1B01), and the Innovation Team Development of the Ministry of Education of China (IRT0967).

REFERENCES

1. **Bentley, D. R., et al.** 2008. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* **456**:53–59.
2. **Feng, L., et al.** 2008. A recalibrated molecular clock and independent origins for the cholera pandemic clones. *PLoS One* **3**:e4053.
3. **Ferenci, T., et al.** 2009. Genomic sequencing reveals regulatory mutations and recombinational events in the widely used MC4100 lineage of *Escherichia coli* K-12. *J. Bacteriol.* **191**:4025–4029.
4. **Li, H., and R. Durbin.** 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**:1754–1760.
5. **Makarova, K., et al.** 2006. Comparative genomics of the lactic acid bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **103**:15611–15616.
6. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
7. **Sun, Z., et al.** 2010. Identification and characterization of the dominant lactic acid bacteria from kurut: the naturally fermented yak milk in Qinghai, China. *J. Gen. Appl. Microbiol.* **56**:1–10.
8. **Titgemeyer, F., R. E. Mason, and M. H. Saier, Jr.** 1994. Regulation of the raffinose permease of *Escherichia coli* by the glucose-specific enzyme IIA of the phosphoenolpyruvate:sugar phosphotransferase system. *J. Bacteriol.* **176**:543–546.
9. **van de Guchte, M., et al.** 2006. The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. *Proc. Natl. Acad. Sci. U. S. A.* **103**:9274–9279.